After Gibson Assembly/In Fusion Cloning

- Thaw competent cells on ice…cells must be on ice and are very delicate. Do not leave off ice for longer than a few seconds

- Add 2 ul of Gibson Assembly reaction to 25-50ul of competent cells. Do not pipette up and down, this will damage the cells. Simply flick the tube a few times to mix and immediately put back on ice.

- Incubate on ice for 10-20 minutes.

- Heat shock the cells for exactly 30 seconds at 42C. It is better to use the water bath in Ian's lab but you can also use the dry bath in the iGEM lab.

- After the heat shock, place cells immediately back on ice for 2-3 minutes

- Add 250 ul of room temperature or warm SOC to the cells
-
- Incubate for 45 min – 1 hour on a shaking incubator at 37C

- Warm LB plates with appropriate antibiotic during the incubation so they will be at least at room temperature by the time the 1 hour incubation is over

- Plate all of the reaction on the LB plates and incubate upside down in a 37C incubator overnight